

1. (Currently amended) An attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein or a functional modification [thereof] of said protein, wherein said duplex region [is a non-chimeric duplex region, but] has [at least] one or two base-pair substitutions such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype.

2. (Currently amended) The virus of claim 1, wherein said virus exhibits a reduction in plaque titre compared to the parent wild-type virus on one or more type of cells selected from the group consisting of Madin-Darby bovine kidney (MDBK) cells, Madin-Darb[u]y canine Kidney (MDCK) cells and Vero cells.

3. (Currently amended) The virus of claim [Error! Reference source not found.] 2, wherein said virus exhibits at least about one log reduction in plaque titre compared to the parent wild-type virus on MDBK cells.

4. (Currently amended) The virus of claim [Error! Reference source not found.] 2, wherein said virus exhibits at least about a 3 to 4 log reduction in plaque titre compared to the parent wild-type virus on MDCK cells and Vero cells.

5. (Previously presented) The virus of claim 1, wherein said genomic nucleic acid segment is a mutated native influenza virus genomic RNA segment.

6. (Currently amended) The virus of claim 1 [or 5], wherein said virus is an attenuated influenza virus of type A and said nucleic acid segment is a mutated influenza A virus genomic RNA segment having a mutation C to A at position 11 from the 3'-terminus of the native parent segment and a mutation G to U at position 12' from the 5'-terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an attenuating base-pair substitution in the non-coding duplex region.

7. (Previously presented) The virus of claim 6, wherein said nucleic acid segment further comprises a mutation U to G at position 10 from the 3' terminus of the native parent segment and a mutation A to C at position 11' from the 5' terminus of the native parent

segment, or functionally equivalent substitutions at the same positions, so as to provide an additional base-pair substitution in the non-coding duplex region.

8. (Currently amended) The virus of claim [7] 6, wherein said nucleic acid segment encodes neuraminidase (NA) or a functional modification thereof.

9. (Previously presented) The virus of claim 1, wherein said virus is a wild-type virus which has been attenuated by said base-pair substitution(s).

10. (Previously presented) The virus of claim 1 further comprising a heterologous coding sequence capable of being expressed in target cells.

11. (Previously presented) The virus of claim 10, wherein said heterologous coding sequence encodes an antigenic peptide or polypeptide capable of stimulating an immune response to a pathogenic agent.

12. (Currently amended) The virus of claim [9] 6 or claim 7, wherein said [wild-type] virus is an attenuated influenza A/WSN/33 and wherein said nucleic acid segment encodes neuraminidase (NA) [having a NA-encoding nucleic acid segment] or a functional modification thereof.

13. (Currently amended) A nucleic acid [sequence comprising] consisting of a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein or a functional modification [thereof] of said protein, wherein said duplex region [is a non-chimeric duplex region, but] has [at least] one or two base-pair substitutions such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype.

14. (Currently amended) [The nucleic acid sequence of claim 13, wherein said sequence is a] A DNA sequence capable of transcription to provide a [RNA sequence] nucleic acid according to claim 13.

15. (Previously presented) A plasmid comprising the DNA sequence of claim 14.

16. (Previously presented) A ribonucleoprotein (RNP) complex comprising the nucleic acid of claim 13 complexed with polymerase proteins and nucleoprotein of an influenza virus.

17. (Currently amended) An ex vivo cell infected by an attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein or a functional modification [thereof] of said protein, wherein said duplex region [is a non-chimeric duplex region, but] has [at least] one or two base-pair substitutions such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype.

18. (Currently amended) A vaccine comprising [comprising ] an attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza viral protein or a functional modification [thereof] of said protein, wherein said duplex region [is a non-chimeric duplex region, but] has [at least ]one or two base-pair substitutions such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype.

19. (Currently amended) The vaccine of claim 18 [further comprising a second pathogenic agent other than an influenza virus] wherein said attenuated influenza virus additionally comprises a heterologous coding sequence which encodes an antigenic peptide or polypeptide capable of stimulating an immune response to a pathogenic agent.

20. (Previously presented) A pharmaceutical composition comprising the virus of claim 10 in combination with a pharmaceutically acceptable carrier or diluent for delivery of said heterologous coding sequence to target cells.

21. (Previously presented) A pharmaceutical composition comprising cells infected with the virus of claim 10 in combination with a pharmaceutically acceptable carrier or diluent.

22. (Currently amended) A method of preparing an attenuated influenza virus comprising the steps of:

constructing a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein or a functional modification [thereof] of said protein, wherein said duplex region [is a non-chimeric duplex region, but] has [at least] one or two base-pair substitutions such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype;

providing to a host cell said genomic nucleic acid segment together with the additional genomic nucleic acid segments for said virus under conditions whereby said segments are packaged into a viral particle; and

selecting said virus.

23. (Currently amended) A method of preparing an [attenuated] influenza virus by a helper virus based influenza gene rescue system comprising the steps of:

transfecting a host cell with a helper virus, wherein said helper virus is an attenuated influenza virus comprising a genomic nucleic acid segment which comprises 5' and 3' non-coding regions providing a mutated duplex region of an influenza virus RNA genomic segment operably-linked to a protein coding sequence for an influenza viral protein or a functional modification [thereof] of said protein, wherein said duplex region [is a non-chimeric duplex region, but] has [at least] one or two base-pair substitutions such that expression of said protein-coding sequence in cells infected by said virus is reduced to give an attenuated phenotype;

transfecting said host cell with a nucleic acid segment of an influenza virus to be rescued, wherein [said host cell contains said helper virus] said nucleic acid segment also encodes said protein or a functional modification thereof; and

selecting viruses containing said nucleic acid segment on the basis of increased growth compared with the helper virus on cells of a selected type.

24. (Currently amended) The method of claim 23, wherein said helper virus is an attenuated influenza A virus and said genomic nucleic acid segment and said nucleic acid segment encode neuraminidase or a functional modification thereof.

25. (Currently amended) The method of claim 24, wherein said helper virus is attenuated influenza A/WSN/33 having in its NA-encoding genomic nucleic acid segment a mutation[s ] C to A at position 11 from the 3'-terminus of the native parent segment [and], a mutation G to U at position 12' from the 5'-terminus of the native parent segment [and], a mutation[s] U to G at position 10 from the 3' terminus of the native parent segment and a mutation A to C at position 11' from the 5' terminus of the native parent segment, and wherein said host cell is a Vero cell.

26. (Currently amended) A method of stimulating an immune response against an influenza virus, [optionally together with stimulation of an immune response against one or more pathogenic agents], comprising the step of administering to an animal in an immunizing mode the attenuated influenza virus of claim 1.

27. (Previously presented) A method of delivering a heterologous coding sequence to cells comprising infecting said cells with the virus of claim 10.

28. (Currently amended) The method of claim 23, wherein said nucleic acid segment of an influenza virus is complexed with [polymerase] polymerase proteins and nucleoproteins to form a RNA complex which is transfected.

29. (Currently amended) The nucleic acid [sequence] of claim 13, wherein said genomic nucleic acid segment is a mutated native influenza virus genomic RNA segment.

30. (Currently amended) The nucleic acid [sequence] of claim 13, wherein said nucleic acid segment is a mutated influenza A virus genomic RNA segment having a mutation C to A at position 11 from the 3'-terminus of the native parent segment and a mutation G to U at position 12' from the 5'-terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an attenuating base-pair substitution in the non-coding duplex region.

31. (Currently amended) The nucleic acid [sequence] of claim 30, wherein said nucleic acid segment further comprises a mutation U to G at position 10 from the 3' terminus of the native parent segment and a mutation A to C at position 11' form the 5' terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an additional base-pair substitution in the non-coding duplex region.

32. (Currently amended) The nucleic acid [sequence] of claim [31] 30, wherein said nucleic acid segment encodes neuraminidase (NA) or a functional modification thereof.

33. (Previously presented) The ex vivo cell of claim 17, wherein said genomic nucleic acid segment is a mutated native influenza virus genomic RNA segment.

34. (Previously presented) The ex vivo cell of claim 17, wherein said virus is an attenuated influenza virus of type A and said nucleic acid segment is a mutated influenza A virus genomic RNA segment having a mutation C to A at position 11 from 3'-terminus of the native parent segment and a mutation G to U at position 12' form the 5'-terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an attenuating base-pair substitution in the non-coding duplex region.

35. (Previously presented) The ex vivo cell of claim 34, wherein said nucleic acid segment further comprises a mutation U to G at position 10 from the 3' terminus of the native parent segment and a mutation A to C at position 11' from the 5' terminus of the native parent segment, or functionally equivalent substitutions at the same positions, so as to provide an additional base-pair substitution in the non-coding duplex region.

36. (Currently amended) The ex vivo cell of claim [35] 34, wherein said nucleic acid segment encodes neuraminidase (NA) or a functional modification thereof.

37. (Previously presented) The ex vivo cell of claim 17, wherein said virus is a wild-type virus which has been attenuated by said base-pair substitution.

38. (Currently amended) The ex vivo cell of claim 17 wherein said virus further [comprising] comprises a heterologous coding sequence capable of being expressed in target cells.

39. (Previously presented) The ex vivo cell of claim 38, wherein said heterologous coding sequence encodes an antigenic peptide or polypeptide capable of stimulating an immune response to a pathogenic agent.

40. (Currently amended) The ex vivo cell of claim [37] 34 or claim 35, wherein said [wild-type] virus is an attenuated influenza A/WSN/33 and said nucleic acid segment encodes neuraminidase (NA) [having a NA-encoding nucleic acid segment] or a functional modification thereof.

Claims 41-47 (Canceled)

48. (New) The virus of claim 6 wherein said genomic nucleic acid segment is a mutated NS1-encoding genomic nucleic acid segment.

49. (New) The virus of claim 6 wherein said genomic nucleic acid segment is a mutated PA-encoding genomic nucleic acid segment.

50. (New) The virus of claim 6 wherein said genomic nucleic acid segment is a mutated PB2-encoding genomic nucleic acid segment.

51. (New) The nucleic acid of claim 30 which is a mutated NS1-encoding genomic nucleic acid segment.

52. (New) The nucleic acid of claim 30 which is a mutated PA-encoding genomic nucleic acid segment.

53. (New) The nucleic acid segment of claim 30 which is a mutated PB2-encoding genomic nucleic acid segment.

54. (New) The ex vivo cell of claim 34 wherein said nucleic acid segment is a mutated NS1-encoding nucleic acid segment.

55. (New) The ex vivo cell of claim 34 wherein said nucleic acid segment is a mutated PA-encoding genomic nucleic acid segment.

56. (New) The ex vivo cell of claim 34 wherein said nucleic acid segment is a mutated PB2-encoding nucleic acid segment